

DATA EVALUATION RECORD
Potential Biological Effects on Freshwater Aquatic Ecosystems Based on its Applications to
Outdoor Microcosms
USEPA Guideline: N/A (Non-guideline)

PC Code: 124002

1. Chemical: Novaluron; (Rimon 10 EC); (1,3-chloro-4-(1,12-trifluoro-2-tri fluoromethoxyethoxy) phenyl]-3-(2,6-defluorobenzoyl) urea

2. Test material: Rimon 10 EC (9.5% ai w/w)

3. Citation:

Author: Jenkins, W. R., et, al. 2002.

Title: "Novaluron ("Rimon" 10EC): Assessment of its Potential Biological Effects on Freshwater Aquatic Ecosystems Based on its Applications to Outdoor Microcosms.

Study Completion Date: April 6, 2002

Laboratory: Huntingdon Life Sciences Ltd., Woolley Rd, Alconbury, Huntingdon, Cambridgeshire, PE284HS, England

Sponsor: Makhteshim Chemical Works Ltd., P.O. Box 60, Beer-Sheeva 84100, Israel.

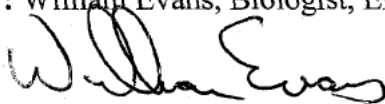
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4. REVIEWED BY: William Evans, Biologist, ERB1, OPP/EFED

Signature:



Date: May 6, 2004

5. STUDY PARAMETERS:

Test Type: Outdoor Microcosm Study

Duration of Test: 147 days after treatment

Application Rate: 0.5, 1.5, 5.0, 15.0, and 50.0 g ai/ha (0.05, 0.15, 0.5, and 1.5 µg/L)

6. CONCLUSIONS: The data from this microcosm study performed at environmentally relevant novaluron concentrations, show reductions in aquatic invertebrate populations, some of the taxa affected never recovered for weeks following initial pesticide exposure. Study results indicate that Novaluron exhibited water column DT₉₀ values ranging from 12 to 20 days for three different test concentrations (i.e., 5, 15, and 50 g a.i./ha treatment level). Only low concentrations of novaluron were detected in sediment, demonstrating potential for microbial degradation. This was confirmed by the presence of the main degradate, chlorophenyl urea (275-352I), in the water column of three out of five tested concentration and in soil of the highest tested concentration. Chlorophenyl urea (275-352I) was the only degradate analyzed in water and sediment.



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Results from the microcosm study suggest that populations of benthic invertebrates can be significantly reduced (up to total eradication in the case of Gammaridea) in aquatic systems where sediment concentrations of novaluron are below levels of detection (detection limit 5 ng/g). Observed impacts to some members of the benthic community were not followed by population recovery, suggesting either low potential for recruitment of new individuals or residual activity of novaluron when partitioned to sediments. Sediment toxicity data, which demonstrate the measured toxicity of novaluron in the sediment and interstitial water, would enable a more quantitative analysis of novaluron risks to sediment dwelling organisms, provided that protocols allow for exposure durations sufficiently lengthy to encompass multiple life stages of the tested organisms.

The data indicate that significant impacts to aquatic zooplankton and benthic macroinvertebrates can occur at nominal initial water concentrations of 0.05 to 5 ug/L. This range of concentrations encompasses all peak, 21-day and most 60-day (except the potato scenario involving ground application) 1 in 10 year return frequency estimates of novaluron in surface waters. Effects in this microcosm study include but are not limited to:

- Total measured zooplankton populations exhibited negative responses to novaluron, as compared to controls for all dose levels, with statistically significant population reductions ($p < 0.01$) at the 0.15 through 5 ug/L dose levels (NOAEC 0.05 ug/L);
- Statistically significant ($P < 0.01$ or $P < 0.05$) reductions in population indices (NOAEC < 0.05 ug/L) for the following zooplankton taxa: Cyclopoidae (recovery after 84 days at this dose level), Chaoboridae (recovery by day 70), and Chirocephalidae;
- Statistically significant ($P < 0.01$ or $P < 0.05$) reductions in population indices (NOAEC 0.15 ug/L) for the following zooplankton taxa: Chydoridae, Lecanidae, and Diaptomidae;
- Benthic invertebrate community response (taxonomic response weighting) show statistically significant ($p < 0.05$) community level effects at the 0.15 ug/L dose level, with a community level response NOAEC of 0.05 ug/L; and
- Complete eradication of all Gammaridae amphipod crustacean populations, at all novaluron concentrations tested NOAEC < 0.05 ug/L.

Finally, the study data indicate that environmentally relevant novaluron concentrations, even below the level of detection, can produce severe and long-lasting impairment of populations of benthic invertebrates.

7. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: There is no current EPA protocol or guidance documents for performing, reviewing, or validating microcosms, the results from this study can only be used as supplemental information in a risk assessment

C. Repairability: N/A

Attached is an abbreviated review of the subject microcosm study entitled "Novaluron ("Rimon" 10 EC): Assessment of its Potential Biological Effects on Freshwater Aquatic Ecosystems Based on its Application to Outdoor Microcosms" submitted by Makhteshim-Agan of North America, Inc. Since there is no current EPA protocol or guidance documents for performing, reviewing, or validating microcosms, the results from this study can only be used as supplemental information in a risk assessment.

8. General Information on Chemical:

Novaluron is an insect growth regulating insecticide in the benzoylphenyl urea family which acts on the pest larval stage by inhibiting chitin biosynthesis and blocking the cuticle formation in target pests. It is currently registered for ornamental use in greenhouse and shadehouses. The current registration application is for a Section 3 use on pome fruit, cotton, and potatoes. Specific information about this proposed registration is presented below.

For food use the registrant proposed two novaluron formulations: 7.5% water dispersible granule (RimOn 7.5WDG) insect growth regulator for control of insect pests on apples and pears and 10% emulsifiable concentrate (RimOn 10EC) insect growth regulator for use on cotton and potatoes. Application by chemigation is prohibited for all proposed uses.

The insecticide should be foliage applied to apples and pears by conventional ground sprayer or airblast sprayer at increased pressure and high volume. According to the proposed label no more than 0.96 lbs a.i. (RimOn 7.5WDG) may be applied per acre per season, with the maximum rate per application 0.32 lbs a.i., the minimum intervals of 10 to 14 days between applications, and up to 3 applications per season are allowed. No application should be made within 14 days of fruit harvest.

RimOn 10EC should be applied to cotton via conventional ground or aerial sprayer, or via band application when cotton is small. The maximum proposed application rate is 0.27 lb a.i. (RimOn 10EC) per acre per season, with the maximum rate per application of 0.09 lbs a.i., the minimum intervals of 7 to 14 days between applications, and up to 3 applications per season. There are currently no instructions on the label concerning the band width and its corresponding application rate within the band or the width of the untreated areas between the rows. No application should be made within 30 days of cotton harvest.

RimOn 10EC should be applied to potatoes by conventional ground spray or aerial equipment. The maximum proposed application rate is 0.23 lb ai/A per season, with the maximum rate for a single application rate of 0,078 lb ai/A. Up to three applications per season could be applied at intervals of 10 to 14 days between applications.

9. Purpose and Objectives of Study:

"The purpose of this study was to assess the potential biological effects of novaluron in invertebrate communities in aquatic ecosystems by analysis of species abundance and diversity in outdoor freshwater microcosms. The study was designed to define the no-effect and ecologically acceptable loading (g ai/ha), an the corresponding no effect concentration and ecologically acceptable concentration (EAC; $\mu\text{g ai/L}$) for novaluron, and to provide information on its fate under natural conditions."

10. Methodology

The design for this system was based on the Society of Environmental Toxicology and Chemistry (Guidance Document, (July 1991), the Organisation for Economic Co-operation and Development Draft Guidance Document, July 1996, and the proceeding of the CLASSIC (Community Level Aquatic System Studies - Interpretation Criteria) workshop (draft June 2001). The test was conducted from 20 March 2001 at the first quantitative sample collection (six weeks before treatment) to 25 September 2001, 147 days after the first application.

11. Test System

The system consisted of 12 satellite ponds surrounding a central reservoir of approximately 127 m³. The central reservoir served as a source for water, aquatic macrophytes and invertebrates for the microcosms. The ponds and reservoir were buried in the ground to minimize temperature fluctuations. The central reservoir was constructed of concrete blocks

with a butyl rubber liner. Each individual satellite pond was divided into four sampling quadrants. These quadrants were used to identify the approximate location of cylinders in each microcosm that were used to measure surface application rates, and for locating a sampling grid that was divided into 25 cm² squares for, 1) determination of the approximate distribution of macrophytes; 2) identify locations for water and sediment depth, and 3) for suspension of monitoring probes and invertebrate sampling equipment. Meteorological conditions were monitored using an automatic weather station.

The central reservoir was established in 1999 by adding a mixture of sediment from a mature untreated pond known to be fish-less, but with invertebrates and macrophytes and local predominately clay top soil. The source water obtained from a licensed abstraction source via a de-chlorination carbon column. To promote primary productivity the reservoir was dosed with nitrogen (0.05 mg/L) and phosphorous (0.3 mg/L) on six occasions (August 3, 8, 15; September 26; October 13; and February 6). Additionally, the microcosms were dosed on February 6 before sampling began on March 20, 2001. The Bur Reed (*Sparganium erectum*) became the dominant species.

The microcosms were established in September 2000. Stainless steel containers were used (diameter 2.7 m, capacity 5.7 m³) with approximately 0.6 m³ of clay based sediment transferred by the use of an excavator to give an approximate depth of 10 cm in each microcosm. Bur Reeds were removed from the reservoir and replanted in an area of one quadrant in a westerly position in each microcosm. In addition, a diverse macrophyte population from the reservoir was transferred from the central reservoir. Invertebrates were also transferred and by sweep netting on October 5 and 10.

Natural sediment was collected from an unpolluted site in Barrow-on-Trent and divided into 24 containers, transferred to each microcosm, and allowed to stand for seven days before leveling with a rake. Sediment samples were analyzed for pesticide residues, and dry matter content, water content, particle size distribution, pH, cation exchange capacity, organic carbon, cadmium, chromium, copper, lead, nickel, and zinc.

Since the site was devoid of trees, a variety of tree leaves were collected and added to the central reservoir and microcosms on six occasions between November 1 and December 6, and then once again on February 6, 2001. On each occasion approximately 150 g dry weight were added to each microcosm.

12. Pre-treatment Phase

During the pre-treatment phase, water and sediment depth were measured on April 12, 2001. In addition, water samples were analyzed for zooplankton populations, chlorophyll a, suspended solids and TOC. Measurements for ammonia, nitrite, nitrate, total oxidised nitrogen, phosphorus, alkalinity, conductivity, total hardness, chloride, sulphate, calcium, iron, manganese, potassium, and sodium six weeks, two weeks, and one day before the application. Sediment samples were analyzed during the week before application for acid soluble metals, pesticide residues, and particle size distribution, organic carbon content, cation exchange capacity, dry matter content, water content and pH. Half the volume of water was pumped from each microcosm to the reservoir then returned to ensure similarity of water quality and zooplankton populations weekly from week six and two. Finally, benthic invertebrates were identified and counted six and one week before treatment.

13. Treatment Phase

Nominal duplicate concentrations of 0.05, 0.15, 0.5, and 1.5 µg/L were randomly applied to 8 of the 12 microcosms as Rimon 10 EC (9.5% ai w/w). A nominal concentration of 5.0 µg/L was applied to one other microcosm and the remaining three microcosms were not

treated and served as controls. These applications were made on May 1, 2001. A second application was made 14 days after (May 15) the first application at the same rate to the same microcosms. Prepared applications were sprayed on the surface of the microcosms using compressed air as propellant and a lance fitted with a single nozzle.

These duplicate nominal concentrations were the equivalent application rates of 0.5, 1.5, 5.0, 15.0, and 50.0 g ai/ha. These rates are equivalent to 0.0027, 0.0081, 0.027, 0.081, and 0.27 lb ai/A.

High Performance Liquid Chromatography (HPLC) was used to analyze novaluron sprayed on the surface of each microcosm at the time of application. Liquid Chromatography- Mass Spectrometry (LC-MS) was used for estimation of surface water application rate as well as the estimation of novaluron and the major degradate CPU in water and sediment.

14. Post-treatment Phase

Water temperature was measured at a depth of 45 cm and monitored continuously throughout the experiment. Conductivity, pH, turbidity and concentration of dissolved oxygen at weekly intervals until Day 133 and then on Day 147 (the last day of the test). Total Organic Carbon (TOC) and suspended solid samples were taken from the same quadrant for each microcosm for analysis every 14 days. Water characterization samples were taken every four weeks after the application until test termination. Characterization included the following measurements: ammonia, nitrite, nitrate, total oxidized nitrogen, phosphorus, total phosphorus, alkalinity, conductivity, suspended solids, total hardness, chloride, sulphate, calcium, iron, manganese, potassium, sodium, and TOC. Sediment was analyzed for acid soluble metals (cadmium, chromium, copper, lead, nickel, and zinc), particle size distribution, organic carbon content, cation exchange capacity, and pH at test termination. Sediment microbial biomass was measured the day before application and 16 weeks after first treatment.

Phytoplankton productivity was estimated the first, second, third, and forth weeks after application, and thereafter, every two weeks through week 16. Productivity was estimated by analysis of chlorophyll a and phaeophytin a in aliquot samples.

The plant communities growing on submerged surface (periphyton) were sampled bi-weekly beginning 4 weeks after application and continuing through week 16. Ash-free dry weight was used to determine biomass measurements.

Zooplankton diversity and abundance was assessed in samples taken on Day 7 after the first application, then three, seven, and fourteen days after the second application and thereafter at 14-day intervals. Zooplankton were identified to family, unless considered to be important in defining the response to novaluron. In those cases the zooplankton were identified to genus and species.

Macroinvertebrate productivity was assessed using colonizers and sediment grab samplers. Macroinvertebrates were identified to family. In addition, a survey of the benthic invertebrates was conducted at the end of the study (Day 147). The numbers of Gammarids were determined from randomly selected microcosms from the controls and each treatment up to the 15 g ai/ha treatment.

Insect emergence traps were installed in all microcosms at the day of application. Collection bottle were renewed each week, and later identified to Order and family as appropriate.

The determination of the disappearance time (DT_{90}) from the water column following the first and second applications. was calculated from samples taken from a depth of 10 cm at

the 5, 15, and 50 g ai/Ha treatment levels.

The assessment of diversity and abundance of invertebrates was accomplished by comparing the controls to treatment levels using the Shannon-Weiner index (Magurran 1988). Statistical analysis of the number of organisms and taxa present from samples taken was carried out using multivariate and univariate analysis due to the inherent complexity and variability of microcosm data.

Temporal changes in the communities and individual taxa was accomplished using taxon count and analyzed by Redundancy Analysis (RDA). A Principal Response Curve (PRC) was then performed as an RDA where the taxon weight and C_{dt} were determined. Community level responses on each sampling occasion were also analyzed using the RDA method. Counts of taxa were then analyzed using Principal Component Analysis (PCA). Differences between the control group and treatment group employed the Williams' test to identify the $NOEC_{community}$.

Responses of individual taxa on each sampling occasion was accomplished by counting the numbers of individuals in each taxon and transformed and individually analyzed using the Williams' test in order to identify the $NOEC_{taxon}$.

The Ecologically Acceptable Concentration (EAC) was defined "as the concentration at or below which no ecologically adverse effects would be expected (HARAP, 1998)". This was based on the impact and recovery from novaluron treatment at the community level.

15. Reported Results

The levels of novaluron in the water column from LC-MS analysis taken one hour after the first application at the 45 cm indicate that levels were below the limit of quantitation (0.05 $\mu\text{g/L}$) for the 0.05 $\mu\text{g/L}$ application rate. The levels found for the 0.15 $\mu\text{g/L}$ rate at the 10 cm depth did not differ greatly from the levels found at the 45 cm depth (0.15, 0.13, and 0.19 $\mu\text{g/L}$). One hour after the second application the levels were in two of the microcosms of at the 0.05 $\mu\text{g/L}$ rate and one microcosm at the 0.15 $\mu\text{g/L}$ rate were verified to be at their respective levels. CPU, the major degradate, was not detected in any of the water sample, and neither novaluron or CPU was detected in the sediment samples.

At the 0.5, 1.5, and 5 $\mu\text{g/L}$ rates both novaluron and CPU were detected at and above these concentrations in all microcosms after the first application, and all above (100 - 180%) nominal concentrations after the second application. These concentrations reduced considerably with the passing of days after both the first and second applications. Percent reductions dropped from 32% in 3 days at the 5 $\mu\text{g/L}$ level to 100% in 13.9 days at the 0.5 $\mu\text{g/L}$ level after the first application. Percent reductions after the second application ranged from 42% in 3 days to either 99% (or below detection limits after 21 - 70 days at all the 0.5, 1.5, and 5 $\mu\text{g/L}$).

Water quality measurements including temperature, dissolved oxygen, pH, conductivity, turbidity, total organic carbon, and suspended solids indicated that no treatment related effects in water quality were observed in the study.

Levels of phytoplankton chlorophyll a and phaeophytin were analyzed to measure primary productivity. In the first six weeks before treatment, levels of chlorophyll a were relatively low (0.03 - 1.79 $\mu\text{g/L}$). In the four-week period before first treatment, chlorophyll a showed cyclical change ranging from 0.63 to 11.73 $\mu\text{g/L}$. Phaeophytin-a were low throughout (<0.24 $\mu\text{g/L}$). Fourteen days after the second treatment chlorophyll a levels (5.19 - 22 $\mu\text{g/L}$) were generally higher than controls (1.36 - 3.73 $\mu\text{g/L}$). Chlorophyll-a levels were generally higher in treated microcosms until Day 84 when compared to controls. Phaeophytin-a levels during

the post-treatment showed occasional increases but were generally low ($<0.485 \mu\text{g/L}$).

Estimates of periphyton biomass was variable with levels ranging from 8.5 to 425.1 mg/m^2 six weeks before treatment and 4.1 to 96.9 mg/m^2 one day before application. After treatment the levels showed a general increase with Day 28 and 42 with the highest level (521.4 mg/m^2). Periphyton chlorophyll-a levels were low in samples taken six weeks, two weeks and one day before treatment and levels were $<0.1 \text{ mg/m}^2$. These levels remained low after treatment.

Macrophyte populations were dominated by Long Styled Starwort (*Callitriche platycarpa*), the Bur-reed (*Sparganium sp*) and the Curled Pondweed (*Potamogeton crispus*). The most diverse microcosms included the Rigid Hornwort (*Ceratophyllum demersum*), the Bulbous Rush (*Juncus bulbosa*), the Pink Water Speedwell (*Veronica catenata*), and *Ranunculus petatus*. A blanket species (*Spirogyra sp.*) completely covered the sediment in 3 microcosms including one control, and as much as possible was removed the day before the first application. This species re-established by May 22, 2001, and to minimize the impact on the weed was collected, placed in onion bags, and returned to the microcosms.

Zooplankton diversity comprised a total of 22 families (9 crustaceans, 5 rotifers, one hydrozoan, and 7 insect larva). The number of taxa ranged from 4 - 9 six weeks prior to the first application and 10 - 14 in the day before application. The number of taxa gradually fell after treatments at the 0.15 , 1.5 , and $5 \mu\text{g/L}$ levels until day 42, then increase to similar levels found in the controls by Day 98. The number of taxa at the $0.5 \mu\text{g/L}$ level were inconsistent.

With respect to individual taxa, the most abundant crustaceans were the larval forms Daphniidae, Chydoridae, Podocopa, and Diaptomidae. The Daphniidae were present throughout the establishment and post treatment phases of the study with sufficient abundance for population assessment in regard to the impact and recovery after treatment. The dominate species was *Daphnia longispina*. Signs of recovery for Daphniidae populations ranged from 7 to 84 days for the 0.15 , 0.5 , 1.5 , and $5 \mu\text{g/L}$ levels. There was no effect at the $0.05 \mu\text{g/L}$ level. Populations of Chydoridae, Podocopa, and Diaptomidae were relatively low before and after treatment, but increased after Day 28.

Rotifer populations were dominated Brachionidae, Lecanidae, and Synchaetidea and all showed increases after treatment at the 0.5 , 1.5 , and $5 \mu\text{g/L}$ level.

Multivariate and univariate analysis of zooplankton indicated cyclical increases that were either synchronous with control population changes or asynchronous. Many populations peaks, declines, or high degrees of variability in numbers throughout the study. Principle Response Curve (PRC) analysis showed concentration-dependent deviation from controls after treatment. Populations were affected at all application rates. Recovery to the control levels was only evident for the 0.05 and $0.15 \mu\text{g/L}$ levels at Day 56 and Day 84, respectively.

The effects at the community level was analyzed by comparing the times after treatment at which changes in population were statistically significant when compared to the controls. The $\text{NOEC}_{\text{zooplankton}}$ was $0.05 \mu\text{g/L}$ and the ecologically acceptable concentration was $0.5 \mu\text{g/L}$ based on an 84 day recovery.

Analysis of the effects on the individual zooplankton level show that statistically significant effects were found on only six taxa and the nauplii on days 84 and 112.

A total of thirty-seven families representing benthic invertebrates on colonizers comprised of two crustaceans, twenty insect larvae, eight round or flat worms, and seven molluscs. During the pre-treatment phase the numbers of taxa in each microcosm ranged from 9 to 16

and the mean number of invertebrates in pooled samples ranged from 64 to 312. In the post-treatment phase the number of taxa were generally similar in all microcosms, but the number of invertebrates found during the test were lower at the 1.5 and 5 $\mu\text{g/L}$ levels.

The benthic crustacea were represented by the Asellidae and Gammaridea families. The members of the Asellidae were found to be comprised entirely of *Asellus aquaticus* and members of the Gammaridea family were exclusively *Crangonyx pseudogracilis*. The populations of Asellids gradually increased from 12 to 40/sample 42 days before treatment to 56 to 193/sample at test termination. Samples taken after treatment were generally reduced below control levels in the microcosms treated at and above 0.5 $\mu\text{g/L}$. The numbers of Gammaridea were variable during the study in the controls. Gammarids numbers were low in all treated microcosms in samples taken after the second treatment. At the end of the study on 720 individuals were found in the control while 8 and 1 were found at the 0.005 and 0.015 $\mu\text{g/L}$ levels, respectively.

Baetidae (mayflies) appeared to peak seven days before the first treatment then declined 28 days after treatment. After Day 70 larva numbers generally increased in controls and the treated 0.005, 0.5, and 1.5 $\mu\text{g/L}$ levels while the numbers were consistently low at the 0.15 and 5 $\mu\text{g/L}$ levels.

The larvae of biting midges (Ceratopogonidae) were found in relatively low numbers. There appears to be no treatment related effects. Damselfly, and caddis fly larvae were present in samples taken throughout the study, and again, no treatment related effects could be identified. Chironomids (non-biting midges) were present in high numbers in colonizer traps and sediment samples. High numbers of larvae were found at the 0.005 and 0.5 $\mu\text{g/L}$ levels. Water boatmen and other aquatic beetle numbers were too low to evaluate. Water mites (Limnocaridae) were found in small numbers during the pre-treatment phase, but increased 28 days after treatment, but the lowest number were found at the 5 $\mu\text{g/L}$ level. Molluscs were occasionally found in individual microcosms in high numbers. Leeches appeared to be generally higher in treated microcosms than controls. The increase in numbers of molluscs and leeches may have been due to the increase in the primary food source for these organisms (periphyton) as well as the treatment-related loss of arthropod grazers.

Multivariate and univariate of benthic invertebrates were analyzed for temporal changes, effects at the community levels, and effects at the taxon level. Temporal changes were analyzed by PRC and since there were no survivals in all treated microcosms numbers were not included in this analysis. However, other invertebrate populations at the 0.05 and 0.15 $\mu\text{g/L}$ levels were unaffected by treatment. Populations at the 0.5 $\mu\text{g/L}$ were effected after treatment, but showed signs of recovery after Day 49. The 1.5 and 5 $\mu\text{g/L}$ treatments showed a decline in the 91 days after the first treatment, but then appeared to stabilize.

The analysis of effects at the community level was identified which changes in populations were statistically significant when compared to controls and shows the NOEC for the benthic invertebrate community was 0.05 $\mu\text{g/L}$ while the EAC was considered to be 0.5 $\mu\text{g/L}$.

Analysis of effects at the taxon level shows the results of comparison in the pre-treatment phase (day -42), in Day 28 when the first effects were observed, and at the end of the test (Day 133). These are summarized in the following table.

Taxon	No-effect treatment Levels ($\mu\text{g/L}$)	
	Day 28	Day 133

Asellidae	0.15	0.5
Cardiidae*	0.50	5.0
Erpobdellidae	1.5	5.0
Gammaridea	<0.05	**
Limnocaridae	5.0	1.5

* numbers were low

** excluded from analysis because none were found in microcosms.

A total of nine taxa were identified in sediment grab samples and included one round worm, four oligochaete worms, one mollusc, and three larval forms of insects. The numbers of taxa were generally similar in all grab samples, and ranged from one to four (occasionally five or six) in a control. The numbers in the appeared to be unaffected by treatment level.

Insects representing a total of fifty taxa were identified in emergence traps. Thirty of these taxa were associated with the aquatic larval stages, and twenty were considered to be terrestrial.

Although initial concentrations of a poorly soluble substance such as novaluron applied to a microcosm is expected to be variable, the measured levels of novaluron in cylinders essentially verified that the application rates coincided with the measured concentrations sampled. The levels ranged from 79 to 125% of the nominal concentrations in 7 of the 10 estimates.

Estimates of the DT_{90} of 18 days (0.5 $\mu\text{g/L}$ level), 12 days (1.5 $\mu\text{g/L}$ level), and 20 days (5.0 $\mu\text{g/L}$ level) indicate that novaluron was not persistent in the water column even about 3 $\mu\text{g/L}$, the water solubility of novaluron. This was attributed to degradation, partitioning into environmental compartments such as sediment and aquatic plants, and volatilization. However, appreciable levels of novaluron were not found in sediments samples in this study by LC-MS analysis. This led the author to suggest that the loss of novaluron from the water was attributed to degradation and partitioning to aquatic plants. The detection of the major degradate chlorophenyl urea (CPU) also confirmed that novaluron was degraded under natural conditions. After the second application of novaluron CPU levels increased, appeared to plateau, then decreased to the end of the test.

Sediment microbial biomass were variable and appeared to unaffected by the treatment regime. Carbon levels generally declined from 139.03 to 272.75 $\mu\text{g C/g}$ at the beginning to between 83.54 to 215.42 $\mu\text{g C/g}$ on the last sample day (Day 112) presumably due to degradation and processing of leaves and detritus by micro-organisms and benthic invertebrates.

16. Reported Conclusions

- Treatment with two applications of novaluron at nominal water concentrations of 0.05, 1.5, 0.5, 1.5, and 5.0 $\mu\text{g/L}$ caused no effect on the physico-chemical properties or primary productivity to the microcosms used in this study.
- The results of LC-MS analysis confirm that the application rates were achieved. The mean times for 90% disappearance (DT_{90}) at 0.5 and 1.5 $\mu\text{g/L}$ subsequent to the second application

were 18 and 12 days, respectively. At 5.0 µg/L, above the limit of solubility (3.0 µg/L) the DT₉₀ was 20 days.

- Degradation of novaluron indicated by the presence in water of its main degradate, chlorophenyl urea (CPU) was readily established in all treated microcosms. Novaluron and CPU were only found occasionally in sediment samples and there was no evidence to indicate the persistence in this compartment of either the parent molecule or its degradate.
- The NOEC of novaluron on the zooplankton community was 0.05 µg/L. Recovery was first evident at 0.5 µg/L 70 days after the first treatment with full recovery to control levels by Day 84, so this was considered to be the EAC
- Significant effects on nauplii and Cyclopoids were observed at the treatment level but the late seasonal development of these populations and complexity of their life cycles suggests that this result is not ecologically significant.
- In the benthic invertebrate community, the Gammarida (*Crangonyx pseudogracilis*) were impacted at all treatment levels but due to the isolated nature of the microcosms and the entirely aquatic life history of this species, recovery was not possible during the study. This lack of recovery may be considered as an artefact of the study design (and not ecologically significant), and hence was not included in the determination of the NOEC for the benthic invertebrate community.
- The NOEC for the benthic invertebrate community was 0.05 µg/L. At 0.15 and 0.5 µg/L communities were not significantly different from controls 49 and 70 days after the first treatment; the EAC was considered to be at the 0.5 µg/L treatment level.
- Significant effects on the Asellidae were observed at the 0.5 µg/L treatment, with recovery 112 days after the first treatment.
- The overall EAC for novaluron based on multivariate statistical analysis of zooplankton and benthic invertebrate community responses was determined to be at the 0.5 µg/L treatment level (initial nominal water column concentration: 0.05 µg/L. This was based on effects and subsequent recovery following two applications.

10. Reviewer's Conclusions:

It is important to note that the nominally exposed chambers at 5 µg/L exhibited measured initial concentrations of novaluron in excess of the solubility limit. Because the samples were unfiltered collections, measured concentrations in excess of solubility may reflect a combination of dissolved and colloidal/particulate associated novaluron in the water column.

Zooplankton measurements included measures of taxa numbers, initially at the family level with a subsequent analysis of discrete genera and species for those organisms defining system response to novaluron. In general, total measured zooplankton populations exhibited negative responses to novaluron, as compared to controls for all dose levels, with statistically significant population reductions ($p < 0.01$) at the 0.15 through 5 µg/L dose levels. Individual taxonomic family responses are summarized as follows:

Taxa	NOAEC for Population Reductions µg/L
Chydoridae	0.15 (day 84)
Cyclopoidae	<0.05 (recovery after 84 days at this dose level)
Nauplii	<0.05 (recovery after 84 days at this dose level)
Synchaetidae	0.5 (day 84)
Daphnidae	> 5.0 (significant reductions in 2 nd phase)

Lecanidae	1.5 (significant increases in numbers at day 84)
Brachionidae	5.0 (significant increases in numbers at day 84)
Chaoboridae	<0.05 (day 42, recovery by day 70)
Chirocephalidae	<0.05 (day 56)
Diaptomidae	0.15 (day 56)

Analysis of benthic invertebrate populations were conducted primarily at the family level, with subsequent analysis to more refined taxonomic levels for those organisms showing definitive responses to novaluron treatment. Analysis of benthic invertebrate community response (taxonomic response weighting) shows statistically significant ($p < 0.05$) community level effects at the 0.15 ug/L dose level, with a community level response NOAEC of 0.05 ug/L. It should be noted that the Gammaridea showed statistically adverse response ($p < 0.01$) below that observed for the community as whole, with a NOAEC <0.05 and complete eradication of the family at all dose groups by study termination.

It is important to realize that all NOAEC's from this study are presented in terms of the initial nominal novaluron concentration. However, many of the effects observed in the study progress over considerable time periods following initial novaluron application. Concurrent with the emergence of observable effects over the course of the study, measurements of water column concentrations of novaluron are declining with time. Consequently the study cannot provide definitive information on the actual water concentration over time that can be associated with an observed adverse effect. Reliance on the nominal concentrations for establishment of NOAECs likely underestimates the toxic potential of novaluron.